JACC Vol. 19, No. 1
January 1992:174–85

REVIEWS

Fish Oils in the Prevention of Atherosclerosis

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The hypothesis that oils derived from the flesh of fish and marine mammals inhibit the atherosclerotic process is critically reviewed. Populations consuming a diet rich in fish have low rates of coronary heart disease. Dietary fish oil is associated with changes in serum lipids, prostaglandin and leukotriene metabolism, enhanced endothelial function and effects on growth factors released from platelets, leukocytes and endothelial cells. Dietary fish oil supplementation has been associated with inhibition of atherosclerosis experimentally induced by dietary hyperlipidemia and balloon injury. Results of studies of the use of fish oil to inhibit postangioplasty restenosis in human subjects have been inconclusive.

(J Am Coll Cardiol 1992;19:174–85)

In the past decade remarkable advances have been made in the treatment of coronary artery disease, particularly in relation to the advanced thrombotic complications of atherosclerosis. Whereas basic research is providing greater insight into the pathophysiology of atherosclerosis itself, to date our progress in preventing and treating the early atherosclerotic lesion has been less spectacular. In this review we will examine the basic, experimental and clinical evidence concerning the hypothesis that fish oils may prevent or retard the development of atherosclerosis.

Support for a positive role for fish oils in inhibiting atherosclerosis is based on four lines of evidence:

1. *Epidemiologic studies* on the effect of fish consumption on morbidity and mortality from coronary heart disease in humans.
2. *In vitro studies* showing that fish oils alter biochemical processes implicated in atherogenesis.
3. *Animal studies* showing an inhibitory effect of fish oil supplementation on the development of atherosclerosis.
4. *Studies with clinical end points in humans.*

Epidemiologic Evidence

Much of the interest in the possible role of fish oils in preventing atherosclerosis derives from epidemiologic observations of Greenland Eskimos. Despite the average 60-year life span of these Eskimos, they had only a 3.5% mortality rate from coronary heart disease (1,2). Original investigations (3,4) showed that the Eskimos, compared with Danish subjects, had low blood levels of cholesterol and triglycerides but elevated levels of high density lipoprotein (HDL) cholesterol despite a very high intake of dietary fat.

Fatty acid content of fish and marine mammals. Analysis of the Eskimos' diet showed that most of the fat and calories were derived from the flesh of oily fish and marine mammals. Biochemically the fatty acid profile of fish and marine mammals differs from that of vegetables or land animals. There is a high content of long chain fatty acids containing 20 or 22 carbons with up to 5 or 6 unsaturated carbons. Furthermore, in fatty acids common in vegetable fat, the last unsaturated carbon is usually located sixth from the methyl end, whereas in fatty acids common in fish, the final unsaturated site is most commonly located third from the methyl end. Thus, fatty acids common in fish have been termed N3 or omega-3 fatty acids, whereas vegetable fatty acids are called N6 or omega-6 fatty acids (Fig. 1). The two most abundant N3 fatty acids found in fish are a 20-carbon fatty acid with five unsaturated carbons called eicosapentaenoic acid and a 22-carbon chain fatty acid with six unsaturated sites called docosahexaenoic acid (Fig. 1). Although many of the important biochemical effects of N3 fatty acids discussed here have been associated with eicosapentaenoic acid, docosahexaenoic acid may also be important. Docosahexaenoic acid tends to concentrate in human phospholipid and can be slowly metabolized to eicosapentaenoic acid. Thus, it may serve as a depot form of eicosapentaenoic acid and perhaps has other, as yet unknown, specific functions. In addition, docosahexaenoic acid inhibits platelet aggregation in vitro and may contribute to the overall platelet inhibitor actions of N3 fatty acids.

Effects of fish and whale meat on platelets and blood cholesterol. Bang and Dyerberg (5) reported that it was common for Eskimos to consume 400 to 500 g of fish or whale meat daily containing up to 7 g of N3 fatty acids and that dietary N3 fatty acids, particularly eicosapentaenoic acid, were incorporated into the phospholipid of the Eski-

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Manuscript received November 7, 1990; revised manuscript received May 30, 1991, accepted October 9, 1991.

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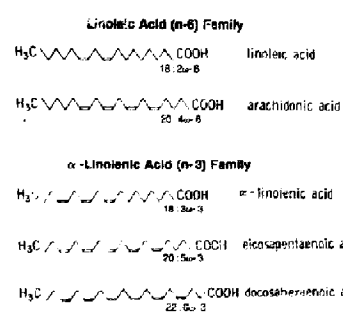

Figure 1. Structural formulas for the N3 and N6 families of fatty acids. Linoleic acid, derived from vegetable sources, undergoes elongation and desaturation in its conversion to arachidonic acid **(top of panel)**. Alpha-linolenic acid is synthesized by plankton and serves as the precursor for eicosapentaenoic acid and docosahexaenoic acid **(bottom of panel)** that are concentrated in the marine food chain. The two families differ in the length of the carbon skeleton, the number of unsaturated bonds and the position of the last unsaturated bond. Marine fatty acids have their first unsaturated carbon third from the methyl end and are thus termed N3 or omega-3 fatty acids; vegetable fatty acids have their first double bond between the sixth and seventh carbon from the methyl end and are termed N6 or omega-6 fatty acids. Reprinted with permission from von Schacky C. Prophylaxis of atherosclerosis with marine omega-3 fatty acids: a comprehensive strategy. Ann Intern Med 1992;107: 890–9.

mos' platelet membranes leading to a prolonged bleeding time and decreased in vitro platelet aggregation (6,7). The low incidence of ischemic heart disease was attributed to inhibition of platelet function and low blood cholesterol levels that were dietary rather than genetic in nature, because Eskimos on a standard Danish diet had serum lipid values similar to those of the Danes (4).

This hypothesis is supported by other epidemiologic studies. As reported by Keys (8), there is a low incidence of coronary heart disease in Japan, where per capita fish consumption averages 100 g daily (9). The inverse relation between coronary heart disease and fish consumption in Japan appears to be dose dependent (9).

A strong inverse relation between coronary heart disease mortality and fish consumption was also found in the Zutphen study (10). However, protective effects correlated with a much lower average daily fish consumption and did not appear to be dose dependent. Compared with non-fish eaters, men who consumed an average of only 30 g/day of fish had a 50% decrease in coronary mortality.

A reexamination of epidemiologic data from the Western Electric study (11,12) also detected an inverse relation between fish consumption and coronary heart disease mortality and showed no positive association between fish intake and mortality from other causes. Despite these results, other studies have failed to demonstrate any protective effect of fish consumption on mortality due to coronary heart disease (13,14).

Summary of epidemiologic data. In perspective, the epidemiologic data provide suggestive, but inconclusive, evidence that fish consumption protects against coronary deaths. Considerable caution is advisable when interpreting these data. For example, it is possible that the striking absence of atherosclerosis in Greenland Eskimos is attributable, at least in part, to genetic rather than dietary influences. For example, although adoption of a western diet by Greenland Eskimos results in adverse changes in their serum lipid profiles, no follow-up data show that this population then experiences a higher incidence of atherosclerotic disease. Studying a different Eskimo population, Rabinowitch (15) believed that he had shown that Canadian Eskimos living in more southerly latitudes (and consuming a more western diet) did show clinical evidence of atherosclerosis. However, this conclusion was based on a greater frequency of sclerotic radial and temporal arteries, as detected by palpation, and a higher mean blood pressure than found in Canadian Eskimos living in more northerly latitudes and consuming a more traditional diet. It is difficult to account for the very positive results of the Zutphen study (10) in a population whose mean consumption of 20 g/day of lean fish would supply only 0.4 to 0.5 g of N3 fatty acids daily. It is probable that the variable results of the different epidemiologic studies may be explained by genetic differences in the study populations, differences in the amount and the type of fish consumed and differences in study design and accuracy of reporting. It should be recognized that there may be serious weaknesses in epidemiologic studies, particularly when investigators use dietary recall methods or patient questionnaires to estimate average daily fish consumption over long periods of time and then attempt to relate this information to coronary events, sometimes without adjusting for the effect of other relevant risk factors.

In Vitro Studies

Fatty acids released from cellular membrane phospholipids function as precursors for many biologically significant products. When fish fat is a major component of the diet, eicosapentaenoic and docosahexaenoic acids are incorporated into the membrane lipids of platelets, leukocytes, erythrocytes and endothelial cells. Because of their structural similarity to arachidonic acid, these fatty acids may provide an alternative substrate for the enzymes cyclooxygenase and lipoxygenase and perhaps others, leading to the synthesis of distinct prostanoids with properties very different from those that are derived from arachidonate (Fig. 2). Thus, it is not surprising that fish oils have diverse biologic effects.

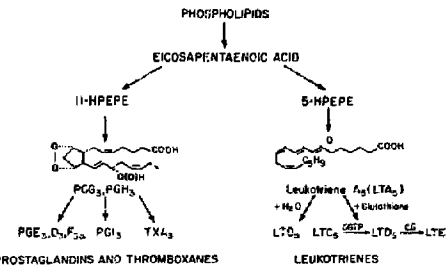

Figure 2. Eicosanoid products of the metabolism of eicosapentaenoic acid. When ingested in the diet, eicosapentaenoic acid is incorporated into platelet, leukocyte and endothelial cell membrane phospholipid and leads to the biosynthesis of a family of trienoic prostaglanoins (PG) including thromboxane (TX) A_3 and prostaglandin I_3, and tetraenoic leukotrienes (LT) including leukotriene B_5. See text for details. Adapted with permission from von Schacky C. Prophylaxis of atherosclerosis with marine omega-3 fatty acids: a comprehensive strategy. Ann Intern Med 1987;107: 890–9.

Effects on prostaglandin metabolism (Fig. 2). As mentioned, Dyerberg and Bang (6) first demonstrated that dietary eicosapentaenoic acid is incorporated into platelet membrane phospholipid by performing chemical analysis on platelets obtained from Eskimos and Danish control subjects. Compared with Danes, Eskimos with a diet rich in fish had decreased in vitro platelet aggregation and a longer bleeding time, findings reproduced in other studies (16,17) and demonstrated to be dose dependent (18). Since then, studies using platelets isolated from volunteers fed a diet rich in fish oil have shown that the eicosapentaenoic acid incorporated into the platelet membrane is converted to thromboxane A_3 (19–22) (Fig. 2), a structural analogue of thromboxane A_2 with no platelet agonist activity (23,24). In endothelial cells, eicosapentaenoic acid is converted into prostaglandin I_3, a trienoic analogue of prostacyclin (25,26). Unlike the biologically inert thromboxane A_3, prostaglandin I_3 retains potent vasodilator and platelet antagonist properties.

Effect of N3 fatty acid on human prostaglandin metabolism. Knapp et al. (22) assessed the effects of N3 fatty acids on prostaglandin metabolism in atherosclerotic subjects and normal control subjects using doses that approximate those ingested by Eskimo populations (10 g/day eicosapentaenoic acid). Baseline urinary excretion of thromboxane A_2 and prostacyclin metabolites was significantly elevated in atherosclerotic subjects, probably reflecting increased platelet-vessel wall interactions and generalized platelet activation (27). Excretion of urinary metabolites of thromboxane A_2 decreased markedly both in patients with atherosclerosis and in healthy subjects, whereas the excretion of urinary metabolites of prostacyclin was significantly depressed only in subjects with atherosclerosis. Increases in urinary metabolites of thromboxane A_3 and prostaglandin I_3 were noted in both groups. Thus, supplementation with fish oil normalized elevated prostacyclin synthesis in subjects with atherosclerosis, suggesting a decrease in abnormal platelet-vessel wall interactions and partially impaired the synthetic capacity for thromboxane A_2 in all subjects. Thromboxane A_2 synthesis was only partly suppressed, probably because eicosapentaenoic acid competes inefficiently with arachidonic acid for cyclooxygenase (25). Recent data showing an increase in platelet survival time in hyperlipidemic patients with documented atherosclerosis consuming a diet supplemented with fish oil provide further evidence that fish oil may cause a decrease in abnormal platelet-vessel wall interactions (29). Thus, there is evidence that high doses of dietary fish oils engender a net change in the hemostatic balance, and thus would appear to protect against thrombosis.

Effects on leukotriene metabolism (Fig. 2). Current evidence suggests that leukotrienes contribute to the acute inflammatory response produced by vascular injury and myocardial infarction (30). In neutrophils and monocytes, membrane-bound arachidonate is converted through intermediates to a family of leukotrienes including leukotriene B_4, a potent chemoattractant for neutrophils and monocytes. Leukotrienes C_4, D_4 and E_4 (31), synthesized by monocytes, have been implicated in coronary vasoconstriction (32), ischemic myocardial depression (33) and ventricular arrhythmia (34).

Role of dietary N3 fatty acids in leukotriene metabolism. In experimental animals exogenous administration of dietary eicosapentaenoic acid suppresses the formation of leukotriene B_4 in a dose-dependent manner and results in the synthesis of a biologically much less active molecule, leukotriene B_5 (35,36) (Fig. 2). Incorporation of eicosapentaenoic acid into neutrophil membrane phospholipid and in vitro generation of leukotriene B_5 have also been demonstrated in humans after dietary supplementation with N3 fatty acids (37,38). Using neutrophils collected from normal subjects ingesting 3.2 g of eicosapentaenoic acid daily, Lee et al. (39) demonstrated in vitro synthesis of leukotriene B_5 and 48% suppression of leukotriene B_4 production. Monocyte synthesis of leukotriene B_4 was also decreased by 58% after 6 weeks. Even the moderate doses of fish oil used in their study led to major impairment in the neutrophil chemotactic response to leukotriene B_4, and to decreased neutrophil adhesion to endothelial monolayers pretreated with leukotriene B_4. Monocyte chemotaxis may also be impaired by fish oils (40).

Although the significance of these findings is speculative at present, altered leukotriene metabolism could potentially have important modulating effects in myocardial infarction and possibly chronic atherogenesis.

Role in myocardial infarction. Neutrophil adhesion to endothelium may be noted within 15 to 20 min of coronary occlusion (41). There is evidence that neutrophil-derived mediators produce alterations in microvascular tone, promote interstitial edema and the "no reflow" phenomenon and contribute to myocardial stunning and arrhythmogenesis. Although the mechanism remains to be clarified, pretreatment with N3 fatty acids in experimental coronary occlusion and reperfusion in dogs, cats and rats (42–44) resulted in significantly smaller infarcts, fewer arrhythmias and fewer sudden deaths than in control animals. Similar results were obtained in a feline carotid artery occlusion model (45); cats pretreated with N3 fatty acids had significantly smaller cerebral infarcts (7% versus 19% of the territory at risk). In addition, dietary tuna oil was protective against ventricular fibrillation during both ischemia and reperfusion in a rat model of coronary occlusion (46). Protective effects have not been associated with impaired thrombogenesis (42,43) or differences in regional myocardial blood flow (42,43) and it is possible that altered leukotriene metabolism and impaired leukocyte responses may have contributed in part to the beneficial effects noted.

Role in atherogenesis. The important role of leukocytes and leukocyte-derived growth factors in atherosclerosis is being increasingly recognized. In the hypercholesterolemic primate model (47) monocytes adhere to endothelium, penetrate the intima and begin to accumulate lipid within 2 weeks of beginning an atherogenic diet. Continued cholesterol feeding results in further monocyte accumulation and smooth muscle cells migrate from the media toward the intima where they proliferate and synthesize connective tissue (47,48). The myointimal proliferation that follows is dependent on many factors including mitogenic and chemotactic factors derived from monocytes, platelets, endothelial cells and smooth muscle cells, as well as toxic release products from macrophages. The overall effects of N3 fatty acids on monocyte/macrophage participation in atherosclerosis remains to be clarified, but it is known that dietary fish oil impairs monocyte chemotaxis (40) and decreases synthesis of interleukin-1 and tumor necrosis factor (discussed later), platelet-activating factor (49) and toxic oxygen-derived free radicals (50).

Effects on Growth Factors

In addition to the effects on prostaglandins and leukotriene metabolism noted previously, N3 fatty acids may affect the synthesis of growth factors by platelets, endothelial cells and monocytes.

Platelets. Platelets synthesize and release several growth factors during their adhesion to the injured blood vessel wall. These include platelet-derived growth factor, epidermal growth factor, transforming growth factor beta, platelet factor 4 and beta-thromboglobulin. Platelet factor 4 and beta-thromboglobulin are chemotactic for smooth muscle cells and monocytes. During the platelet-vessel wall interaction stimulated by vascular injury, platelet factor 4 rapidly penetrates the intima and media and may play an important contributing role in the subsequent hyperplastic response (51). In a study by Hay et al. (52), administration of 3.5 g of eicosapentaenoic acid daily to 13 patients with ischemic heart disease led to a 75% decrease in blood levels of platelet factor 4 and a 70% reduction in beta-thromboglobulin, whereas platelet survival time (29.52), a marker of platelet activation and consumption, was increased by 10%. A decrease in serum beta-thromboglobulin was also shown by Knapp et al. (22) after dietary supplementation with high dose eicosapentaenoic acid. Neither study elucidated the mechanism whereby fish oil led to decreased blood levels of these substances. To date, no study has examined the effect of dietary fish oil on release of platelet-derived growth factor from platelets, although there is evidence regarding release of this mitogen from endothelial cells.

Endothelial cells. Endothelial cells are known to synthesize mitogens for vascular smooth muscle cells and fibroblasts (53), including platelet-derived growth factor (54). Recent in vitro evidence (55) has demonstrated nearly complete suppression of endothelial cell platelet-derived growth factor activity when cultured bovine endothelial cells were incubated with fish oil concentrate, an effect that apparently depends on oxidative processes because it was inhibited when antioxidants were added to the culture medium. This effect may be important in inhibiting intimal hyperplasia in the setting of denuding endothelial injury as found after angioplasty because endothelial release of platelet-derived growth factor appears to be markedly increased in the setting of endothelial injury or cell death (56).

Monocytes. A recent study (57) demonstrated that dietary supplementation with 4 to 5 g of N3 fatty acids daily can suppress the synthesis of interleukin-1 and tumor necrosis factor by monocytes and other mononuclear cells obtained from normal human volunteers. Theoretically, impaired interleukin-1 and tumor necrosis factor release could inhibit atherogenesis in several ways because these substances are known to have a direct toxic effect on the endothelium, promoting leukocyte adherence and inducing a procoagulant state (58,59). In addition, interleukin-1 has direct mitogenic activity for smooth muscle cells (60) and stimulates fibroblast growth and metabolism resulting in increased biosynthesis of collagen and other connective tissue elements (61).

Effects on Blood Lipids

Despite the vast number of experimental animal and human feeding trials, much confusion persists regarding the effects of fish or concentrated fish oil consumption on serum lipids. Two comprehensive recent reviews (62,63) summa-

rize what is currently known, based on human and animal feeding trials.

Triglyceride levels. These are generally decreased in a dose-dependent manner with fish or fish oil supplementation. At very high doses of up to 20 to 30 g/day of oil, fish oils are among the most powerful hypotriglyceridemic agents available, although the effect may not always be sustained (64).

Total cholesterol levels. These are variably affected by fish or fish oil supplementation. Results from animal studies are inconsistent, probably because of differences in the dose of fish oil used, species differences and differences in the remainder of the diet. Similar variability is noted in trials involving humans. In many cases decreases in total cholesterol levels are due to a fall in very low density lipoprotein (LDL)-cholesterol in patients with a marked hypotriglyceridemic response to fish oil.

LDL cholesterol levels. These are variably affected by N3 fatty acids. In trials involving fish or fish oil administration to normolipidemic humans (62,63), the major factor determining its effect on LDL cholesterol has been the amount of saturated fat in the diet. When fish oil was added to the diet (and saturated fat intake held constant), LDL cholesterol levels tended to rise. When fish oil was substituted for saturated fat, LDL cholesterol levels decreased in 30% to 40% of cases and remained unchanged in the rest; this effect is similar in magnitude to that seen when polyunsaturated vegetable oils were substituted for saturated fat (62,63). In studies with hyperlipidemic patients, saturated fat intake was usually not a variable and LDL cholesterol levels rose in most of the studies.

HDL cholesterol levels. High density lipoprotein (HDL) cholesterol levels have been reported to be increased, decreased or unchanged after dietary supplementation with N3 fatty acids. Most placebo-controlled crossover studies (63) have shown an increase in HDL cholesterol of 5% to 10% with small to moderate doses of fish oils; these effects may reflect a reciprocal relation with triglycerides. When very high doses of N3 fatty acids are used, HDL cholesterol may fall and this effect may also be seen with polyunsaturated vegetable oils in high doses. Some studies (65,66) indicate that N3 fatty acids in high doses increase HDL levels with short- but not long-term supplementation. A study in nonhuman primates (67) suggests that when HDL levels are decreased, this effect may be due to the synthesis of smaller HDL particles with a lower cholesterol content per particle. Finally, there is some evidence (68) that N3 fatty acids may increase HDL turnover or up-regulate HDL receptors.

Correlation of inhibitory effects on atherosclerosis and serum lipoprotein concentrations. Although a number of experimental studies showing inhibitory effects of N3 fatty acid supplementation on atherosclerosis failed to correlate these effects with favorable changes in serum lipoprotein concentrations, it should be noted that N3 fatty acids may alter the atherogenicity of lipoprotein particles by affecting their size, apoprotein content and physical properties (63) or alter their uptake and turnover by affecting receptor mechanisms (68). These effects are potentially clinically relevant and would not be reflected in simple measurements of blood lipid concentration. Concerning the inconsistent results of fish oil feeding on total, LDL and HDL cholesterol concentrations, these reflect very large differences in study design, dosing, patient-related variables and especially the overall dietary composition and total ratio of polyunsaturated to saturated fatty acids. In addition, heterogeneity of the fatty acid composition and cholesterol content of the fish oil supplements themselves may explain some of the variability of effects of fish oils on human lipoprotein levels. Most fish oil capsules contain only 12% eicosapentaenoic acid and 18% docosahexaenoic acid—the remaining 70% of fatty acids are composed of a variable mixture of saturated and monounsaturated and polyunsaturated fatty acids. In a recent study (69), hypercholesterolemic men fed 2.7 g of highly purified eicosapentaenoic acid daily had a major reduction in total and LDL cholesterol with an increase in apoprotein A-I levels, suggesting a change in the composition of the HDL particle.

Effects on Other Relevant Variables

N3 fatty acids also influence a variety of other clinical and laboratory variables relevant to the development of atherosclerosis (Table 1).

Endothelium-derived relaxing factor. It has been demonstrated (70) that dietary supplementation with N3 fatty acids may enhance in vitro endothelium-dependent vasodilation in a dose-dependent manner in hypercholesterolemic and atherosclerotic pigs. Recently the relevance of this effect has been confirmed angiographically in patients with coronary artery disease (71). Patients with significant disease of the left circumflex or right coronary artery but only mild lumen irregularities of the left anterior descending artery had a significant dose-dependent vasoconstrictor effect to acetylcholine injected selectively into the left anterior descending artery, indicating endothelial dysfunction. Patients were retested after 6 months of high dose fish oil administration. Improved endothelial function was noted in all patients, with resumption of a normal vasodilator response in 75%. Similar results were recently demonstrated (72) in heart transplant recipients after dietary supplementation with N3 fatty acids.

Fibrinolytic responses. A reduction in the concentration of plasminogen activator inhibitor-1 levels was found by Mehta et al. (73) in the blood of normal subjects as well as those with coronary artery disease after 4 weeks of dietary supplementation with N3 fatty acids. Although the mechanism of this effect is unclear, it may be related to the hypotriglyceridemic effect of N3 fatty acids. Recently two studies (74,75) demonstrated a fibrinogen-lowering effect of N3 fatty acid supplementation. Although this effect was dose dependent (75), a clinically significant effect (21.6% decrease in fibrinogen) was seen after 20 weeks of supplementation with only a moderate dose of fish oil concentrate supplying 2.2 g/day of N3 fatty acids. Preliminary evidence (76) sug-

Table 1. Effects of N3 Fatty Acids on Blood Cells, Lipids and Coagulation: Clinical Implications

Atherogenesis and Intimal Hyperplasia	Thrombogenesis	Vasomotion	Ischemia and Reperfusion	Inflammation
Platelets				
Dec. factor 4; dec. B-thromboglobin; inc. platelet survival	ec. TXA_2; inc. TXA_3; ... platelet count; inc. ... ing time	Dec. TXA_2; inc. TXA_3		
Endothelium				
Dec. PDGF	Inc. PGI_3; preserved PGI_2; inc. EDRF effect	Inc. PGI_3; inc. EDRF effect		
Monocyte				
Dec. IL-1/TNF; ? dec. chemotaxis; dec. free radical synthesis; ? dec. monocyte-fibrinogen interactions	Dec. PAF		Dec. free radical synthesis; dec. PAF	Dec. LTB_4; inc. LTB_5
Neutrophil				
Dec. LTB_4; inc. LTB_5			Dec. LTB_4; inc. LTB_5; dec. adhesion and chemotaxis	Dec. LTB_4; inc. LTB_5; dec. adhesion and chemotaxis
Lipids				
Variable effects*	Dec. triglycerides			
Coagulation				
Dec. fibrinogen	Dec. fibrinogen; dec. PAI-1			

Dec. = decreased; EDRF = endothelium-derived relaxing factor; IL-1 = interleukin-1; inc. = increased; LTB_4 = leukotriene B4; LTB_5 = leukotriene B5; PAF = platelet-activating factor; PAI-1 = plasminogen activator inhibitor-1; PGI_2 = prostacyclin; PGI_3 = prostaglandin I3; TNF = tumor necrosis factor; TXA_2 = thromboxane A_2; TXA_3 = thromboxane A_3. *Depends on composition of diet and total polyunsaturated to saturated fatty acid ratio.

gests that docosahexaenoic acid may have a more powerful effect on fibrinogen levels and platelet function than that of eicosapentaenoic acid; thus, further research will be needed to elucidate which fatty acids should be given and in which doses to produce optimal inhibition of hemostatic function.

Blood pressure. Some studies (77,78) have shown a mild hypotensive effect of N3 fatty acids that appears to be dose related. Studies (79,80) using low doses of N3 fatty acids (3 to 3.5 g/day) have had inconsistent effects on blood pressure.

Animal Studies

Antiatherogenic effects of N3 fatty acids in pigs and monkeys (Table 2). Experimental studies have examined the potential antiatherogenic effects of N3 fatty acid supplementation in several animal species, with the most notable positive results in pigs and monkeys. Clear decreases in coronary lesion area in hyperlipidemic swine given fish oil supplements have been observed (70,81,82). A relatively low dose of cod liver oil (30 ml/day) was added to an atherogenic diet in each of these studies. In two studies (70,81) balloon abrasion of the left anterior descending artery was also performed to stimulate lesion development. Each study showed a significant decrease in coronary lesion area assessed morphometrically in serial sections of the arteries but no apparent beneficial effects on serum lipids. In another study (83), normolipidemic pigs, fed a lard or lard-mackerel diet, had a nonobstructive Teflon constrictor surgically implanted on the left anterior descending coronary artery. After a 4-month period, the pigs were killed and lumen encroachment was evaluated by morphometric techniques similar to those used by others (81,82). Lumen encroachment was increased less in the lard-mackerel oil group (11%) than in the group given lard only (62%).

A follow-up study by this group (84) demonstrated apparent regression of atherosclerosis after N3 fatty acid feeding in the pig model. Coronary atherosclerosis was induced by balloon abrasion of the left anterior descending artery in pigs fed a cholesterol-lard diet for 4 months. After this induction period, control pigs were killed; balloon-abraded arteries had 11% lumen encroachment compared with 1.3% in nonabraded coronary arteries. Other groups of pigs subject to the same induction protocol were then fed diets containing lard, fish oil or both for 3 additional months. After this time, fish oil-fed pigs had significantly less lumen encroachment in the balloon-abraded arteries (3%) than did the control pigs (11%) or those given a lard diet (13%). Lumen encroachment in nonabraded arteries progressed in the lard-fed group (11%) but not in the fish oil-fed group (1.1%).

In rhesus monkeys fed a 2% cholesterol and coconut oil diet for 12 months (85), cholesterol levels rose to approximately 900 mg/dl and up to 80% of the intimal surface of the aorta demonstrated atherosclerotic changes. Monkeys receiving this diet but also receiving supplements of either a low or a high dose of menhaden oil, had a 40% to 55% dose-dependent reduction in the involved aortic intimal surface area despite a corresponding dose-dependent decrease in HDL concentrations. Surface involvement was also decreased in the carotid and femoral arteries. Both cholesterol content and lesion thickness were shown to be decreased by menhaden oil in this study. In another study (86) in hyperlipidemic monkeys, animals with fish oil-

Table 2. Studies of Fish Oil Supplementation in Experimental Atherosclerosis

Ref. (1st author)	Species	Model	Type and Amount of Fish Oil	Effect on Atherosclerosis	Effect on Lipid
Weiner (81)	Pig	Dietary hyperlipidemia and balloon abrasion	Cod liver oil (30 ml/day)	Markedly decreased	Mildly decreased HDL-C, no change LDL-C
Kim (82)	Pig	Dietary hyperlipidemia	Cod liver oil (30 ml/day)	Markedly decreased	Mildly decreased total C, LDL-C
Hartog (83)	Pig	Normolipidemia; Teflon constrictor on LAD	4.5% mackerel oil + 4.5% lard	Mildly decreased	Decrease total C; HDL-C, LDL-C not assessed
Shimokawa (70)	Pig	Dietary hyperlipidemia and balloon abrasion	2% cholesterol + cod liver oil (36 ml/day)	Moderately decreased	No different in LDL:HDL ratio in treated versus controls
Davis (85)	Rhesus monkey	Dietary hyperlipidemia	Menhaden oil (MO) 1. 25% MO/coconut oil 1:1 2. 25% MO/coconut oil 3:1	Mildly decreased Markedly decreased	Mildly decreased total C, markedly decreased HDL-C
Hollander (86)	Cynomolgus	Dietary hyperlipidemia	Fish oil (unspecified) 2 g/day	Mildly decreased; mildly increased at carotid bifurcation	No significant effect
Chamberlain (87)	Japanese quail	Dietary hyperlipidemia	Maxepa concentrate,* 8.6% of diet by weight	Slight increase	Increase total C and LDL-C
Chamberlain (88)	Japanese quail	Dietary hyperlipidemia (generally susceptible strain)	Maxepa concentrate,* 7% of diet by weight	Mildly decreased	Markedly decreased total C
Zhu (89)	NZ rabbit	Dietary hyperlipidemia	Protochol concentrate* 1. Low dose (1 ml/day)	Mildly decreased	Blunted rise in total C, mildly decreased HDL-C
			2. Medium dose (2 ml/day)	Markedly decreased	Blunted rise in total C, mildly decreased HDL-C
			3. High dose (3 ml/day)	Mildly decreased	Increased total C, markedly decreased HDL-C
Bolton-Smith (90)	NZ rabbit	Serum sickness	Fish oil (type unspecified) (20 g/kg)	Mildly decreased	Mildly decreased total C
Thiery (91)	NZ rabbit	Dietary hyperlipidemia	Maxepa concentrate* (2 ml/day)	Markedly increased	No change in total C
Rich (92)	WHHL rabbit	Genetic hyperlipidemia	Maxepa concentrate* (2.5 ml/day)	No change	No change
Sarris (95)	Rat	Heart transplant	Super Maxepa concentrate (2 ml/kg per day) EPA 130 mg/kg per day; DHA 86 mg/kg per day)	Markedly decreased	
Rogers (94)	Rat	1. Dietary hyperlipidemia 2. + Hypothyroidism	Maxepa concentrate (90 mg/kg per day)	Mildly increased foam cells Mildly increased monocyte adhesion and mildly increased foam cells	Mildly increased total C, mildly decreased HDL-C
Landymore (96)	Dog	Autologous grafts and hyperlipidemia	Cod liver oil; EPA (1.8 g/day)	Markedly decreased (intimal thickness)	No difference

*Protochol and Maxepa concentrates supply 18% eicosapentaenoic acid and 12% docosahexaenoic acid by weight. C = cholesterol; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; HDL-C = high density lipoprotein cholesterol; LAD = left anterior descending coronary artery; LDL-C = low density lipoprotein cholesterol; NZ = New Zealand; Ref. = reference number; WHHL = Watanabe Heritable Hyperlipidemic rabbit.

supplemented diets demonstrated a 50% to 66% reduction in the surface area of the aorta, coronary arteries and common carotid arteries with atherosclerotic changes compared with animals not receiving N3 fatty acids. However, there was a significant but unexplained increase in atherosclerosis at the carotid bifurcation.

Studies in lower species. These have yielded conflicting results. Moderate doses of fish oil have had inhibitory effects on atherosclerosis in Japanese quail (87), but these effects were not observed at higher doses in spontaneously atherosclerotic strains (88). In rabbits, studies with different designs have shown a decrease (89,90), increase (91) or no

change (92) in total aortic atherosclerosis with dietary fish oil supplementation. In one study (93), either fish oil or verapamil appeared to cause regression of atherosclerosis in rabbits returned to a normal diet after a period of cholesterol feeding but there was no additive effect when the two agents were combined.

One unusual study in rats (94) examined the effects of fish oil supplementation on monocyte adhesion and foam cell formation. In normocholesterolemic rats supplemented with fish oil, no difference was found in monocyte adhesion or foam cell formation compared with control rats. Rats made hypercholesterolemic by cholesterol and cholic acid feeding exhibited a similar degree of monocyte adhesion, but those whose diet was supplemented with fish oil had a twofold increase in foam cell formation. Another group of rats was made severely hypercholesterolemic by feeding with cholesterol and cholic acid and the addition of the antithyroid agent 2-thiouracil. In this group animals on a fish oil-supplemented diet exhibited a fourfold increase in monocyte adhesion and intimal foam cell density. Although provocative, this effect may be species specific or mediated in part by an interaction with fish oil and hypothyroidism induced by 2-thiouracil. In the previously discussed study by Kim et al. (82) in the pig model, 2% oil-fed animals exhibited significantly fewer monocytes within the atherosclerotic lesions than did the control animals.

Another study (95) showed beneficial effects of N3 fatty acids in the rat cardiac transplant model. Fish oil-fed rats were compared with rats fed safflower oil plus aspirin and dipyridamole and control rats fed a standard laboratory diet. When compared with the other groups, fish oil-fed rats demonstrated fewer diseased vessels and a lower mean arterial disease grade but no difference in transplant rejection, thus suggesting a nonimmunologic mechanism. The safflower oil/aspirin/dipyridamole group showed a degree of atherosclerosis similar to that seen in control rats.

Finally, in a hyperlipidemic canine model, several studies (96–98) have demonstrated that the addition of fish or fish oil to the diet resulted in significantly less intimal hyperplasia in autologous femoral artery vein grafts than that seen in control grafts. Although one study (96) found a similar but smaller protective effect of aspirin and dipyridamole in preventing intimal hyperplasia, another study (98) compared fish oil, aspirin, an experimental thromboxane synthetase inhibitor (CGS-12970) and the combination of fish oil with aspirin or CGS-12970 and found that only fish oil prevented intimal thickening perhaps because of a weak reduction in serum growth factor activity. There was no additive benefit when fish oil was combined with platelet inhibitors. Using synthetic femoral artery vein grafts 4 mm in diameter, Casali et al. (99) demonstrated significantly less neointimal thickening in dogs fed a mackerel diet supplemented with menhaden oil than in dogs consuming a regular diet with or without aspirin and dipyridamole. Graft patency rates were similar in the groups receiving supplemental fish oil and platelet inhibitors and significantly better than those in dogs receiving neither; however, neointimal growth was inhibited only in the dogs receiving the fish diet.

Studies With Clinical End Points in Humans

Postangioplasty Restenosis

Data are now available for analysis from five studies (100–104) of N3 fatty acids in human postangioplasty restenosis. Restenosis was significantly lower in two studies (100,101) and unchanged in two others (102,103). For unexplained reasons, the final study (104) showed a significant limitation in restenosis in a group with single-vessel coronary disease but no difference in patients with multivessel disease. In some ways angioplasty serves as an ideal model for the study of intimal hyperplasia, but it is important to emphasize three concepts before drawing conclusions from these studies:

1. *From molecular biologic study,* it has recently been shown that activation of genes by platelet-derived growth factor occurs within 4 h of angioplasty, ribonucleic acid and protein synthesis begin within 24 h and peak within the 1st week (Taubman F, personal communication). Thus, restenosis represents a healing response to injury that begins immediately.
2. *N3 fatty acids exert many of their effects by becoming incorporated into cellular membranes* in competition with other dietary fatty acids, thus requiring a loading period. A reasonable approach to ensure maximal effectiveness would be to begin the administration of N3 fatty acids about 2 weeks before angioplasty whenever the procedure is elective.
3. *Restenosis rates can only be compared between* studies using a standardized definition of restenosis and with angiographic follow-up. Use of clinical criteria or stress testing may underestimate or overestimate the actual incidence.

Design of postangioplasty studies using N3 fatty acids. Of the postangioplasty studies cited earlier, only two (100,102) included angiographic follow-up in the majority of patients. In the study of Dehmer et al. (100), 82 men with 103 coronary lesions were randomly assigned to receive 3.2 g/day of N3 fatty acids or placebo, in addition to aspirin and dipyridamole. Therapy was begun 1 week before angioplasty and continued for 6 months. Compliance with therapy was assessed by determination of platelet membrane fatty acid composition and restenosis identified by repeat coronary angiography. This study showed a significant decrease in restenosis in the treatment group whether analyzed per lesion (36% vs. 16%) or per patient (46% vs. 19%).

In contrast, Grigg et al. (102) also employed routine angiographic follow-up at 4 months but found no difference in restenosis in patients treated with 1.8 g of N3 fatty acids daily beginning the day before or the day of angioplasty.

Although their study failed to confirm a beneficial effect of N3 fatty acids, the results emphasize the importance of angiographic follow-up in accurately detecting restenosis that was angiographically present in only 51% of patients with recurrent symptoms and in only 55% of those with a positive exercise test. In contrast, 38% of patients with restenosis were asymptomatic. N3 fatty acids were begun before angioplasty in two other studies (101,103); in one (103), there was no benefit, and the other showed a reduction in restenosis from 35% in control subjects to 22% in patients receiving supplements of 4.5 g/day of N3 fatty acids. However, the results of both these trials are weakened because each employed a stepwise follow-up in which repeat angiography was performed only for clinical symptoms or an abnormal exercise test.

Recommendations. Because of the inadequacies in design of the current studies in light of the considerations we have outlined, the present data are inconclusive. The heterogeneous fatty acid composition and cholesterol content of the fish oil supplements used in the various trials may be an additional confounding factor (69). To test adequately whether N3 fatty acid supplementation inhibits postangioplasty restenosis, further randomized, double-blind, placebo-controlled clinical trials will need to be conducted, including studies in patients with both stable and unstable anginal syndromes. Adequately designed trials must allow a sufficient wash-in period for the study drug; this may be assessed by biochemical analysis of fatty acid content of platelet membrane phospholipid. Determination of the restenosis rate should be based on angiographic follow-up.

Secondary Prevention of Myocardial Infarction

Diet and Reinfarction Trial (DART). A very interesting recent trial (105) suggests that small increases in dietary fish intake may prevent secondary mortality after myocardial infarction. In a trial involving more than 2,000 patients, men were advised to increase their dietary intake of fish, increase their dietary intake of fiber or decrease their consumption of total fat while emphasizing the use of polyunsaturated vegetable fat. Because the trial involved a factorial design, all possible combinations of these interventions were tested. After 2 years all cause mortality was 29% lower in the "fish advice" group and this decrease was entirely attributable to a reduction in deaths from ischemic heart disease. Curiously, no decrease in nonfatal ischemic heart disease events occurred in any group. Survival in the "fat advice" group was unchanged despite a small decrease in total cholesterol and an increase in HDL cholesterol. Survival in the "fiber advice" group tended to be worse than in other groups, an effect that was not statistically significant and thus possibly due to chance. Compliance was monitored primarily by dietary questionnaire, although subsets of patients had 7-day weighted dietary intake records and measurements of plasma fatty acids that suggested adequate compliance. This DART trial (105) involved only modest increments in fish intake (about 300 g of fatty fish or 2.5 g of eicosapentaenoic acid weekly) and so corroborates results from the Zutphen (10) and Western Electric (11,12) studies, which also noted beneficial effects of relatively small quantities of fish.

Adverse Effects

Relatively few adverse effects have been related to the consumption of fish or concentrated fish oils. Mild decreases in the platelet count are common but clinically significant thrombocytopenia is very rarely seen, and then only at very high doses (106).

Increased blood sugar in diabetic patients. One study (107) showed an increase in blood sugar levels associated with N3 fatty acids in patients with noninsulin-dependent diabetes. This effect has not been described in patients with insulin-dependent diabetes, in whom fish oil administration has been associated with some favorable metabolic effects, such as partial normalization of the transcapillary albumin escape rate (108). However, pending further study, caution is warranted in advising heavy consumption of either fish or concentrated fish oils in noninsulin-dependent diabetic patients.

Carcinogenic effects. Concerns about the possible carcinogenic effects of fish oils have not been supported by experimental or clinical evidence (106), although some experimental evidence (106) suggests a possible antineoplastic effect.

Contamination with toxins and heavy metals. Finally, in advising a long-term increase in dietary fish consumption, possible contamination with heavy metals and organic toxins is of concern. Recent evidence (109) has documented that fish caught in contaminated waters may be an important source of human exposure to dibenzofurans and dioxins. Further study will be needed to catalogue the levels of these and other toxins in various species of fish and their oils and to assess the biologic consequences to persons who consume large amounts of fish.

Conclusions. Epidemiologic studies provide suggestive evidence that fish consumption may decrease mortality rates from coronary heart disease. This is supported by a wealth of experimental evidence showing a beneficial effect of N3 fatty acid consumption on clinical and biochemical variables implicated in the pathophysiology of vascular disease. Experimental studies of atherosclerosis in pigs and monkeys point to the clinical relevance of the in vitro results but this has not been consistently observed in other animal models. Studies of the impact of N3 fatty acid supplementation on postangioplasty restenosis are inconclusive because of the heterogeneity of study design.

The hypothesis that N3 fatty acids inhibit the atherosclerotic process is sufficiently supported to justify its further study in humans. Although a recommendation may be made for moderate increases in dietary fish consumption on the

basis of the available data, particularly in individuals with known or suspected coronary disease or after myocardial infarction, it is not yet justified to recommend supplementation with concentrated fish oils pending further evaluation of safety and efficacy.

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